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THE MORPHOLOGY AND CYTOLOGY OF THE AECIDIUM CUP

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(WITH PLATES I AND II AND EIGHT FIGURES)

F. D. FROMME

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(WITH PLATES I AND II AND EIGHT FIGURES)

Recent cytological investigations of the Uredineae have materially aided in clearing up the obscure points connected with their complex life history and development. The nature of the sexual processes involved in the transition from the gametophytic to the sporophytic (haploid to diploid) stages has received much attention, and, although the results achieved are somewhat conflicting on minor points, the general nature of the phenomena described by the different authors is quite uniform. Although examples of both the caeoma and cup types of aecidia have been studied from this standpoint, the most clear and acceptable accounts have been given for the less complicated, superficial caeomas. No comprehensive cytological account of the stages in cup-formation or of the general morphology of the aecidium has been given. Certain authors also disagree as to whether the nuclear divisions are all mitotic or in part amitotic.

So far sexuality has been reported in 13 species of rusts; 11 of these cases may be regarded as well established for the present, but the remaining 2, *Uromyces Poae* and *Puccinia Poarum*, as reported by BLACKMAN (6), must be considered as doubtful or at best not entirely understood, since only a few inconclusive fertilization stages were seen. Of the remaining 11 cases, 9 are forms that have aecidia or primary uredosori in their life cycles, and 2 are forms that

lack aecidia. Of the first group, 2 species have aecidia of the cup type and 5 have aecidia of the caeoma type; while the remaining 2 have primary uredosori that have been shown to be functional aecidia. The 2 forms that lack aecidia are short-cycled microforms. Of these 11 species, 10 are autoecious and one (*Melampsora Rostrupi*) is heteroecious.

TABLE I

SUMMARY OF FERTILIZATION PHENOMENA

Species	Author	Date	Length of cycle	Type of aecidium	Method of fertilization
Phragmidium violaceum....	Blackman	1904	o, I, II, III	Caeoma	Nuclear migrations
Phragmidium speciosum....	Christman	1905	o, I, III	Caeoma	Equal cell fusions
Melampsora Rostrupi.....	Blackman and Fraser	1906	o, I, II, III	Caeoma	Equal cell fusions
Phragmidium Pot. canadensis	Christman	1907	o, II^1,II^2,III	Primary uredo	Equal cell fusions
Caeoma nitens..	Olive	1908	o, I (?)	Caeoma	Equal cell fusions
Triphragmium Ulmariae....	Olive	1908	o,II^1,II^2,III	Primary uredo	Equal cell fusions
Puccinia transformans.	Olive	1908	o, III		Equal cell fusions
Caeoma nitens..	Kurssanow	1910	o, I (?)	Caeoma	Equal cell fusions
Puccinia Falcariae....	Dittschlag	1910	o, I, III	Cup	Equal cell fusions
Endophyllum Sempervivi...	Hoffman	1912	o, I	Cup	Equal cell fusions
Melampsora Lini.....	Fromme	1912	o, I, II, III	Caeoma	Equal cell fusions
Puccinia Malvacearum	Werth and Ludwigs	1913	(o?), III		Equal cell fusions

In 10 of the cases reported the fertilization processes described are essentially of the type that involves a complete cell fusion between two equal gametes as originally described by CHRISTMAN for *Phragmidium speciosum*. In the remaining case, *Phragmidium violaceum*, as described by BLACKMAN, two cells are also involved in the fertilization, but these cells were held to be morphologically unequal and complete cell fusion was not observed. Nuclear migrations apparently similar to those described by BLACKMAN, but variously placed in the hyphae, have been reported by CHRISTMAN, KURSSANOW, OLIVE, and the writer, and were interpreted as pathological phenomena. The 10 cases of equal cell fusions are described by 7 different authors, and none of these has found true

fertilizations of the type described by BLACKMAN. OLIVE (22), however, reports and figures cases of fertilizations in *Caeoma nitens* (*Gymnoconia interstitialis*) and *Triphragmium Ulmariae* that suggest the nuclear migrations of BLACKMAN, but are in reality, as he says, simply early stages in cell fusion in which the connecting pore is as yet small. This pore enlarges subsequently and complete union of the cells is accomplished.

A summary as to the fertilization phenomena in the 11 species where fertilization has been described to date is given in table I.

Besides the question of sexuality, there are a number of unsettled points relating to the morphology of the aecidium cup. Some of these questions were raised by DEBARY and have as yet not been completely settled. For example, does the cup enlarge by the addition of new spore chains between those previously formed, or are the new chains added at the lateral borders? Is the peridium formed from the peripheral chains only, or are the central chains also involved? Do buffer cells similar to those found in the caeomas also occur in the aecidium cup?

DEBARY (4) stated that the hymenium of *Aecidium Berberidis* broadens before the cup completely matures, but was unable to determine how this was brought about. He states that the peridium arises from the marginal "basidia," and the entire outer chains composed of peridial cells arched over the center of the cup to form a covering over the central spore chains. RICHARDS (27) found that the growth of the hymenium was largely at the periphery and that only rarely were new chains added between those already formed. He states that the peridium is formed from the apical cells of the central spore chains and from all of the cells of the peripheral chains. The peridial cells are first formed at the center, and they are successively formed on all sides of this center until a complete protective layer is formed over the entire mass of spores. OLIVE (23) agrees substantially with RICHARDS as to the method of formation of the peridium.

The work of RICHARDS, BLACKMAN, CHRISTMAN, OLIVE, KURSSANOW, and others has been summarized by DITTSCHLAG (10), HOFFMAN (15), MAIRE (19), and the author (12), and therefore only the more recent papers will be reviewed.

DITTSCHLAG (10) was the first to describe sexual fusions in an aecidium of the cup type. He found cell fusions at the base of the young aecidium of *Puccinia Falcariae*, but was unable to trace completely the origin of the fusing cells. A single sterile cell was sometimes found above each gamete, but was not always present. Trinucleated basal cells were found that apparently arose from the fusion of three cells, and these basal cells gave rise to chains of trinucleated aecidiospores and intercalary cells. He further described branching basal cells for the first time. The primary basal cell formed a bud on the lateral wall, and two daughter nuclei from the preceding conjugate division passed into the bud. The bud enlarged further and then the nuclei proceeded to divide and give rise to a second chain of spores. His description of the origin of the peridium agrees with that of RICHARDS.

HOFFMAN (15) studied the development of the aecidium of *Endophyllum Sempervivi*, a species that completes its life cycle with the production of a single spore form, an aecidio-teleutospore, besides the spermatia. He distinguished two kinds of tissue in the young cup. The first tissue formed disintegrated to provide room for the development of the spores, and a second tissue, the "Paarungsgewebe," was then formed at the base of the primordium. The cells of this tissue were conspicuous for their size and the density of their cytoplasm, and were borne in filaments that had their long axes parallel to the surface of the leaf. Fertilization was accomplished by the dissolution of the adjoining cell walls between two gametes and perpendicular basal cells resulted. He also found triple cell fusions and trinucleated basal cells and spore chains. He thinks that one of the nuclei in a trinucleated spore eventually disintegrates, but obtained no convincing evidence of this. He also found branching basal cells like those described by DITTSCHLAG, and states in addition that the conjugate nuclear division that precedes the formation of the branch produces two pairs of nuclei of unequal size. The smaller pair enters the branch and the larger pair remains in the original half of the basal cell. His figures of this process are not at all convincing.

The author (12) has described the nuclear development of the

caeoma of *Melampsora Lini*. This form differed in structure from previously described caeomas in that two sterile cells were normally produced above each gamete. Equal cell fusions were found in abundance. A number of three and a few four-cell fusions were found, and these gave rise to chains of three and four-nucleated aecidiospores and intercalary cells. Multinucleated cells, whose origin and fate were not determined, were also found among the spore chains.

TABLE II

PLURINUCLEATED CELLS

Author	Date	Rust	No. of nuclei to a cell	Kind of cell
Sappin-Trouffy	1896	Ur. Betae	4	Uredospore
Blackman and Fraser	1906	P. Malvacearum	3	Teleutospore and basal cell
Blackman and Fraser	1906	Ur. Ficariae	3	Teleutospore
Blackman and Fraser	1906	P. Poarum	3 and 4	Aecidiospore initial cell
Blackman	1904	Ph. violaceum	3	Basal cell
Olive	1908	P. Cirsiil-anceolati	Up to 15	Cell at base of aecidium
Dittschlag	1910	P. Falcariae	3	Aecidiospores and basal cells
Hoffman	1911	Endo. Sempervivi	3	Aecidiospores and basal cells
Fromme	1912	Mel. Lini	3 and 4 3, 4, and 11	Aecidiospores Basal cells

WERTH and LUDWIGS (28) studied the teleutosorus of the microform *Puccinia Malvacearum*. They found cell fusions between the tips of club-shaped hyphae. The fusing cells were usually of unequal size and the nucleus of the smaller passed over into the larger. This binucleated cell cut off two cells, the lower of which became the stalk, and the upper, after a division, the two-celled teleutospore. They occasionally found binucleated cells in the vegetative tissue and were unable to explain their presence.

The occurrence of plurinucleated cells in the rusts is of some interest, since the uninucleated and binucleated condition seems to be very constant in the gametophytic and sporophytic mycelium, respectively. A number of instances of plurinucleated cells that have been figured by various authors are tabulated in table II.

Material and methods

The aecidia included in this study were collected during the spring and summer of 1912 in the vicinity of New York City and at Woods Hole, Massachusetts. Some 20 different species of aecidia were fixed and examined, and the most favorable were selected for a more detailed study.

The 6 species treated here are *Puccinia Claytoniata* Peck on *Claytonia virginica*, *P. Violae* (Schum.) DC. on *Viola papilionacea*, *P. Hydrocotyles* (Link) Cke. on *Hydrocotyle umbellata*, *P. Eatoniae* Arthur on *Ranunculus abortivus*, *P. angustata* Peck on *Lycopus virginicus*, and *Uromyces Caladii* Farlow on *Arisaema triphyllum*. I am indebted to Dr. J. C. ARTHUR for the identification of the aecidia.

Some of these species have been previously studied from the standpoint of sexuality. The aecidium of *Ur. Caladii* has been studied by RICHARDS (27) and by CHRISTMAN (9). RICHARDS has also studied an aecidium on *Ranunculus* that probably belongs to the same species as the one studied here. So far as I have ascertained, the remaining forms have not previously been studied cytologically.

Three of these rusts, *P. Violae*, *P. Hydrocotyles*, and *Ur. Caladii*, are eu-autoecious forms, that is, all four spore forms are included in the life cycle and all are borne upon the same host. Two of the other species, *P. Eatoniae* and *P. angustata*, are eu-heteroecious forms, the former with the uredosori and teleutosori on a grass, *Sphenopolis* (*Eatonia*), and the latter with these sori on a sedge, *Scirpus*. The remaining species, *P. Claytoniata*, is autoecious, but lacks the uredo stage. It belongs to the *opsis* group of SCHROETER'S classification and to the genus *Allodus* of ARTHUR'S (1).

In 3 of the species the aecidia are borne on a mycelium that is diffused throughout the tissues of the host. In the other 3 the mycelium is localized within a rather restricted area. These two types of aecidia can usually be distinguished at a glance. The aecidia from a diffused mycelium are distributed uniformly over the leaf or stem surface at approximately equal distances apart, and all on one part of the host are usually at the same stage of development. When the mycelium is localized, the aecidia are crowded

together in groups, often with an annular arrangement, and the older ones are found in the center of the group and the younger at the margins. This distinction is of some importance in collecting material for study, as it is usually necessary to make several collections of the forms from a diffused mycelium in order that all stages may be represented, while all stages may be present in a single fixation when the aecidia are from a localized mycelium.

All of the material was fixed in the field. Small segments of leaves or stems bearing aecidia were immersed immediately after removal in a small vial of fixing solution. Of a number of fixing solutions tested, weak and medium Flemming's were found to give the best results. The segments were allowed to remain in the fixative for 48 hours, after which they were washed, hardened, and imbedded in paraffin. The sections were stained for the most part with the safranin, gentian violet, and orange G combination, although iron-hematoxylin was used to some extent for comparative study.

The development of the cuplike aecidium

Puccinia Claytoniata proved to be an exceptionally favorable form for study. The cells and nuclei of the fungus are large, and the host tissue is soft and succulent and apparently permits rapid penetration of the fixing solution. The vegetative mycelium that precedes the formation of the spermogonia and aecidia is especially abundant and conspicuous. It is found in all of the leaf tissues, but is most abundant in the mesophyll near the lower epidermis. Most of the hyphae have their axis of growth in the long axis of the leaf. They are almost entirely intercellular except for the haustoria, which are short, knoblike, and usually penetrate only a short distance into the host cell. The hyphae are somewhat irregularly septate. The individual cells vary considerably in size. The average breadth is about 5 μ and the length three or four times the breadth. A single globular nucleus is located, usually, near the center of the cell; its diameter is but slightly less than the short diameter of the cell. The small nucleole stains a ruby red and is readily seen. The chromatin stains a deep blue and is distributed in small globular masses that are connected by delicate strands.

These often appear to be oriented on a point on the nuclear membrane, as OLIVE has found (22), but the presence of a central body was not ascertained with certainty. The hyphae branch monopodially at irregular intervals. The branch usually arises in the lateral wall near the apical end of the cell.

Binucleated cells were sometimes found in the vegetative mycelium, and continuous filaments of these were found in some cases. It is probable that the sporophytic mycelium had already established itself, and that both gametophytic and sporophytic mycelia were associated together in the same leaf tissue.

The aecidia are hypophyllous, and the first evidence of their formation is found in a conspicuous massing of hyphae between the lower epidermis and the cells of the first, second, and third layers of the mesophyll. The center of the young hyphal mass lies between the first and second cell layers. The hyphae are conspicuous on either side of this center for a distance of about two host cells. Thus the area to be occupied includes, in cross-section, 12 host cells, 4 cells broad and 3 cells deep. These host cells are gradually destroyed and replaced by the fungous hyphae.

The host cells in the center of the area are the first to disappear. The hyphae ramify between them, force them apart, and multiply in the intercellular spaces. They act as a wedge between the layers of host cells, and in forcing them apart produce a slight elevation of the epidermis. After these host cells have been completely cut off, they gradually diminish in size, and in most cases completely disappear eventually. The hyphae do not penetrate the host cells during their disintegration, and their disappearance seems to be due to the pressure brought to bear on them by the enlargement of the fungous mass. Their walls become much convoluted and infolded. The host cells are not always completely destroyed; sometimes they may be found imbedded in the tissue of an old aecidium.

In order that they may be described more clearly, the different surfaces of the hyphal mass will be designated as apical, basal, and lateral surfaces, the apical surface being that adjacent to the epidermis. This will apply to either an epiphyllous or hypophyllous aecidium. The individual filaments that make up the young

hyphal mass have their direction of growth toward the center of the mass, a point that usually lies between the first and second layers of host cells. In the very young stages the direction of growth is scarcely evident, since the hyphae wind around the intervening host cells; but as the host cells are crowded out and their site filled by branches of the surrounding hyphae or by others that force their way into the mass, it becomes more apparent. Not all of the hyphae are able to reach the center of the mass. Those on its lateral surfaces are crowded out by the earlier formed hyphae within and grow toward the epidermis, encircling the surface of the hyphal mass. There are 5 or 6 layers of these encircling hyphae.

The cells that make up the hyphal mass of the young cup are shorter and broader than those of the vegetative mycelium. Their cytoplasm is more dense and stains more deeply. This is particularly true of the cells of the interior, while those on the surface of the mass are less sharply differentiated from the purely vegetative cells. As the mass increases in size with the continued destruction of host cells, the fungous cells at the center begin to disintegrate. The first evidence of their disintegration is seen in the appearance of vacuoles in their cytoplasm. This is accompanied by the disorganization of the nucleus. They become detached from the hyphae on which they are borne and round up and enlarge to two or three times their original size if sufficient space is available. Some of these cells evidently undergo complete disintegration, while others become almost entirely empty except for a few cytoplasmic shreds and the remnants of the nucleus, and remain for some time in the center of the mass. They often become closely packed together as a result of enlargement, and have the appearance of a parenchyma. Because of this condition, this tissue of sterile cells is known as the pseudoparenchyma of the young cup (text fig. 1).

The first pseudoparenchyma cells formed are the apical cells of the hyphae that converge at the center of the hyphal mass. The disintegration of the cells of these hyphae proceeds from their apices toward their bases, and the area of pseudoparenchyma is increased by the addition of further cells on its margins. The apical surface of the mass is soon reached, and the sterilization of hyphae then

continues toward the lateral and basal surfaces. The hyphae that run from the basal surface of the mass to its center are on the average about 10 cells in length, while those that run from the lateral surfaces are somewhat shorter. Fig. 1 shows a section through the lateral surface of a young hyphal mass in which the sterilization of 3 or 4 of the apical cells of the hyphae has been accomplished. At the top of the figure the border of an epidermal cell, marking the apical surface of the mass, is indicated by a double line, and a cell of the mesophyll at the base of the figure indicates the basal surface. The cells beneath the pseudoparenchyma are shorter and broader than those on the lateral and basal surfaces of the mass, their

FIG. 1.—Formation of the pseudoparenchyma in the aecidium of *P. Claytoniata:* the attachment of the forming pseudoparenchyma cells (*x*) with the hyphae on which they are borne is evident.

nuclei are larger, with larger nucleoles, and their cytoplasm is considerably denser. Several layers of hyphae at the base of the figure that run tangentially to the curved basal surface of the mass are seen to give rise to the perpendicular hyphae that bear the differentiated, well nourished cells. The perpendicular hyphae shown in the figure average about 6 or 7 cells in length and have apparently cut off 3 or 4 sterile cells each. The sterilization process continues until only 4 or 5 cells at the bases of the perpendicular hyphae remain. The pseudoparenchyma is thus extended until it occupies about two-thirds or three-fourths of the vertical diameter of the mass. The remaining cells of the perpendicular hyphae now stand

out sharply from the pseudoparenchyma and the undifferentiated cells below them. Their cytoplasm is dense, their nuclei large, and the nucleoles especially large and prominent. They are apparently richly nourished at the expense of the cells of the underlying hyphae and vegetative mycelium. These richly nourished cells form a tissue, 2 or 3 cells in depth, that lines the inner basal surface of the young aecidium cup and extends for some distance upward on its lateral surfaces.

A comparison with the caeoma type of aecidium naturally suggests that these richly nourished cells are the gametes, and, as a matter of fact, practically all of the cells of this cup-shaped tissue may later fuse in pairs. Even though the fusion of all of them is not ultimately accomplished, the fusing pairs are distributed throughout the tissue and, before the fusions, those which are to fuse cannot be differentiated from the others.

These gametes of the aecidium cups of *P. Claytoniata* are quite comparable to the gametes of the caeomas. The aecidium of the caeomas is broad and shallow, and the gametes are produced in a single continuous layer beneath the leaf epidermis from which they are separated by a single layer of sterile cells only. They are the penultimate cells of the gametophoric hyphae. The aecidium of *P. Claytoniata* is spherical in shape and is deep-seated in the tissue of the host. The gametes form a tissue, 3 or 4 cell layers in depth, that lines the basal surface of the spherical mass. Above the gametes is found a tissue of sterile cells that has resulted from the disintegration of the upper two-thirds of the gametophoric hyphae. In the cup both the gametes and sterile cells form a tissue, while in the caeoma they form but a single layer. The sterile cells of the pseudoparenchyma and the "buffer" cells of the caeoma seem to be of similar origin and bear the same relation to the respective gametes above which they are borne. In both cases they are possibly potential gametes which have become sterile. If the sterile cells of the cup and the "buffer" cells of the caeoma are in reality homologous, BLACKMAN'S conception, that the latter are morphologically trichogynes, would have to be extended to include a pluricellular type of trichogyne. It may be noted in passing that OLIVE (**22**) contends that the "buffer" cells are sometimes wanting

in *Caeoma nitens* and *Triphragmium Ulmariae*, and that according to my own observations (12) two "buffer" cells are normally produced above each gamete in the caeoma of *Melampsora Lini*. Further, I have found cases of two gametes borne one above the other in the same hypha in this form (12, pl. 9, fig. 18). *Melampsora Lini* thus seems to be an intermediate form between the more simple caeomas, with but one sterile cell and gamete to each hypha, and the aecidium cup with several sterile cells and gametes. It should be mentioned in this connection that the sterile cells of the cup can scarcely be considered to function as "buffer" cells in the sense that CHRISTMAN (7) used the term. If any function is to be ascribed to them, it is apparently that of space making. Their disintegration provides room for the development of the spores.

Up to this point in the development of the cup no multinucleated cells were seen, nor any other large cells that could in any way be considered central cells or organs from which the gametophoric hyphae might have arisen. Careful search was made for them in all stages in all of the forms included in this work, as well as in some additional forms. My observations do not agree, therefore, with those of MASSEE (20) as to the presence of central organs, nor with RICHARDS' (27) as to the presence of a "fertile hypha," nor do I find the multinucleated cells of OLIVE (22). I am confident that these multinucleated cells do not normally occur in any of the forms that I have studied. It is possible that they are peculiar to certain types of aecidia only.

The conditions found in the development of the aecidia of the other species investigated up to the time of the fertilization stage are very similar to those described for *P. Claytoniata*. Excepting minor differences, such as involve the form and size of the cup, its position in the leaf tissue, the relative extent of the pseudoparenchyma, and the number and position of the gametes, the general morphological development of all is of the same nature. The same excavation of the mesophyll of the host in areas of varying size and shape, and the same sterilization of radial hyphae to form the pseudoparenchyma are found in all.

The most noticeable difference found is in the position of the gametes. *P. Violae* is more like *P. Claytoniata* in this respect, and

in the general appearance of the sorus, than any of the others. The number and position of the gametes are practically the same in both. The sterile cells of *P. Violae* do not disintegrate so completely as do those of *P. Claytoniata*, and consequently form a more compact pseudoparenchyma. *P. Violae* is likewise a very favorable form for study, although the later stages do not fix so favorably as do the earlier ones.

In general appearance the early development of *Ur. Caladii* is much unlike that of the two foregoing species. The aecidium is much broader and shallower and is more superficially located in the leaf tissue. The young aecidium has the appearance of a deep-seated caeoma. The perpendicular gametophoric hyphae do not converge toward a central point in the young hyphal mass to such a noticeable degree, but run almost vertically toward the epidermis, a condition to be expected in a broad shallow sorus (text fig. 2). This condition of the gametophoric hyphae is more suitable to the study of the individual hyphae, and the sterilization of their apices and the position of the gametes are more easily seen.

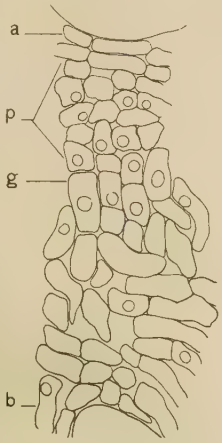

FIG. 2.—Section through the young aecidium of *Ur. Caladii: a*, apical surface; *b*, basal surface; *g*, gametic tissue; *p*, pseudoparenchyma.

The gametophoric hyphae consist of about 6 or 7 cells (text fig. 2). The upper 3 or 4 of these are sterilized and the remaining cells at the base are the gametes. As a rule, about two layers of gametes are formed, though in some cases there was apparently only one, and three were sometimes seen. The gametes are especially large at the time of fusion. The sterile cells do not disintegrate very completely prior to the fusions, and consequently scarcely any characteristic pseudoparenchyma appears. The aecidium is favorable for study, since both cells and nuclei are large. In comparing the sections of my material on *Arisaema* with RICHARDS' figures (**27**, figs. 1–4) of *Ur. Caladii* on *Peltandra*, it seems evident that the aecidium on

Peltandra is more spherical in form and less caeoma-like. My material of the aecidia on *Peltandra* was poorly fixed and only a few sections were cut. It is evident from these, however, that the general shape of the cup here is as RICHARDS has drawn it. A comparative study of the aecidia on these two hosts should be of interest in determining the relative influence of the host tissues on the morphology of the fungus.

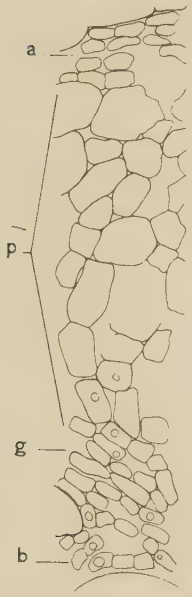

FIG. 3.—Section through the young aecidium of *P. Eatoniae*: *a*, apical surface; *b*, basal surface; *g*, gametic tissue; *p*, pseudoparenchyma.

The aecidia of *P. angustata*, *P. Hydrocotyles*, and *P. Eatoniae* are less favorable for study than any of the foregoing species. The cells of the young aecidia of these forms form a more compact hyphal mass, in which the direction of the individual hyphae is more difficult to trace. The pseudoparenchymatous tissue of all 3 forms is extensive and reaches to the lateral surfaces of the young aecidia, and to a deep point at their basal surfaces. My material of these forms was somewhat old, and the series of early stages is consequently less complete than in the case of the others. The directions of growth of the hyphae and their differentiation into the tissues of the cup are essentially similar to those described for *P. Claytoniata*. The gametes of these forms are not so sharply differentiated from the cells of the pseudoparenchyma and the underlying vegetative tissue, and often cannot be distinguished with certainty except when found fusing.

Since all my material of *P. angustata* was rather old, the gametes or fusion stages were not observed. The location of the two-legged basal cells seen (figs. 26, 27) seems to indicate that the gametes lie at the very bases of the perpendicular gametophoric hyphae. In *P. Eatoniae* the perpendicular hyphae are completely sterilized and the gametes are found in the upper two or three layers of the horizontal hyphae that line the basal surface of the hyphal mass. This

condition is illustrated in text fig. 3. The general direction of the hyphae on the lower border of the figure is tangential to the curved basal surface of the mass. These horizontal hyphae are present in all forms, and, as previously stated, give rise to the perpendicular hyphae. Fusions between cells of these horizontal hyphae are sometimes found in *Ur. Caladii*, but the gametes of this species are normally borne in the perpendicular hyphae. In *P. Eatoniae*, however, the gametes seem to be normally formed in these horizontal hyphae. *P. Hydrocotyles* is more like *P. Claytoniata* in the position of the gametes (text fig. 4). About 5 or 6 cells are found in the perpendicular hyphae below the pseudoparenchyma, and apparently any of the cells in this tissue may function as gametes.

FIG. 4.—Section through the young aecidium of *P. Hydrocotyles*: *a*, apical surface; *b*, basal surface; *g*, gametic tissue; *p*, pseudoparenchyma.

Sexual fusions and spore-production

The fertilization in all these forms is accomplished by the fusion of two closely associated gametes that are normally equal in size and position. The first fusions are found in the central part of the gametic tissue, and the wave of fusions proceeds from this center toward the lateral borders of the aecidium. The fusions are quite abundant, and, though not so readily distinguishable as in the caeomas, many may be found in a favorable section (fig. 23). The process of fusion is identical with that found in the caeomas, and involves the dissolution of an area of the adjoining cell walls between two gametes. The gametes before the fusions are more or less differentiable by their size, the size of their nuclei, and the density of their cytoplasm. The nucleoles are especially large and conspicuous.

The dissolution of the walls may take place anywhere in the

region of contact between the gametes. They usually lie in parallel hyphae with their lateral walls in contact, and when they are in this position and are of equal height, the initial point of dissolution is usually near the middle of the cells or slightly above. A small pore is first formed. Figs. 4 and 5 are of early fusion stages between gametes of *P. Claytoniata*, and fig. 20 between those of *P. Violae*. As a rule, the dissolution of the walls continues on all sides of this pore until practically all of the adjoining walls have been dissolved away. Figs. 6, 16, 17, 21–24 show this condition in various forms. Fig. 23, of *P. Hydrocotyles*, is of especial note since three fusions are shown clearly within a small area. The figure shows further that the fusing cells have no definite position in the hypha or tissue in which they are borne. The two gametes on the right lie immediately beneath the pseudoparenchyma. Those in the center are considerably deeper in the tissue and are the third and fourth cells below the pseudoparenchyma of the respective hyphae in which they are found, while the pair on the left is placed midway between the others. A binucleated basal cell lies above the left gamete of this pair.

The fusions found in *P. Eatoniae* are different from those found in the other forms in that the gametes are located in horizontal hyphae at the base of the cup. A completed fusion between two such gametes is shown in fig. 24, and just above this lies the base of a two-legged basal cell. The upper part of the basal cell has been cut off in sectioning. The right gamete of the basal cell is the adjoining cell in the same hypha with the upper gamete of the fusion cell. Four layers of horizontal hyphae are found between the fusion cell and the host cell that marks the lower boundary of the sorus. According to HOFFMAN (15), the gametes of *Endophyllum Sempervivi* are also formed in horizontal hyphae near the base of the cup, and his figs. 1, 3, and 4 are very similar to corresponding stages in *P. Eatoniae*.

When the fusion of the gametes is completed, the fusion cell elongates in the direction of the epidermis and the nuclei move up into the central part of the cell (figs. 19, 22, 25–27). In *P. Eatoniae*, where the gametes lie in a horizontal position, the elongated part of the fusion cell often turns up at a sharp angle, as in fig. 25. The

cytoplasm of the fusion cells is more dense in the upper and central part and is often vacuolate in the base. For this reason it is often impossible to make out the two-legged character of an old fusion cell. Further, the fusion of the cells is often complete, and this also serves to make the detection of fusion cells more difficult in the cup aecidia than in the caeomas, where the fusion is usually between the tips of the gametes, and the bases remain distinct. After the elongation of the fusion cell, the nuclei divide conjugately in the upper central part. In the early stages of division the two spindles stand out sharply (fig. 10). They lie parallel to each other, with their poles in the long axis of the fusion cell. The process of mitosis was not studied in detail, but it seems evident, from the examination of a number of stages in both gametophytic and sporophytic cells, that the essential features are as described by Olive (22). The spindle figures in the early stages are very small. No central bodies could be differentiated at the poles and radiations were only rarely seen. Fig. 11 shows a late anaphase with the dumb-bell-shaped appearance that has been figured by many investigators of nuclear division in the rusts.

With the completion of the conjugate nuclear division, the fusion cell divides and the apical one-third, containing two daughter nuclei from separate spindles, is cut off (figs. 8, 14). This cell, the so-called aecidiospore mother cell, or, as it should more properly be termed, the aecidiospore initial cell, redivides soon afterward, producing the aecidiospore and the small intercalary cell. The basal cell meanwhile elongates and repeats the process of nuclear and cell division. Fig. 8 shows a distinctly two-legged basal cell of *P. Claytoniata*. Both the basal cell and the aecidiospore initial cell are four-nucleated as the result of conjugate division. The subsequent divisions of the basal cell and aecidiospore initial cell produce a catenulate series of alternating aecidiospores and intercalary cells.

The terms "fusion cell" and "basal cell" are not synonymous as I have used them. The fusion cell is the immediate product of the sexual cell fusion and may or may not function as a basal cell. It becomes a basal cell with the production of aecidiospores. The basal cell is a conidiophore ("basidium" of the earlier writers) from which the spores are abstricted. The term "basal cell" may with

this understanding be used with reference to the conidiophore of any of the spore forms found in the rusts, while the term "fusion cell" should be restricted to indicate the cell in which the transition from the uninucleated to the binucleated condition occurs.

In addition to the normal cell fusions between two gametes a number of cases of triple cell fusions were found. Two of these are illustrated in figs. 7 and 18. Fig. 7 is of *P. Claytoniata* and fig. 18 of *Ur. Caladii*. In both of these cases the three fusing cells lie in separate hyphae. In one or two other cases found two of the gametes came from the same hypha. Similar cases have also been found in *Mel. Lini* (**12**, fig. 18). The trinucleated fusion cells resulting from a fusion of three cells may function as basal cells. In this case the three nuclei divide simultaneously (figs. 12, 13), in the same manner as those in the binucleated fusion cells, and trinucleated aecidiospores and intercalary cells are formed. In fig. 9 a trinucleated aecidiospore initial cell has been abstricted from a trinucleated basal cell. The basal cell appears two-legged, but it is probable that one of the legs has been cut off in the section. Mature trinucleated aecidiospores are quite common in *P. Claytoniata;* several are often found in one section of a mature cup. They are somewhat less common in *Ur. Caladii* and *P. Violae* and are only rarely met with in the other forms.

Quadrinucleated aecidiospores were also found, but in fewer numbers than the trinucleated ones. A chain of quadrinucleated cells is shown in fig. 14. The apical aecidiospore of the chain contains 4 nuclei, while none are visible in the intercalary cell below it. Only 3 nuclei appear in the next aecidiospore, but the subtending intercalary cell is distinctly quadrinucleated. The lowest cell of the chain is probably an undivided aecidiospore initial cell. The 4 nuclei are arranged serially in the long axis of the cell. The chain was cut off at this point in the section and could not be located in adjoining sections. While no four-cell fusions nor four-legged basal cells were found, there can be little doubt that these occur in the cupulate aecidia as well as in the caeoma of *Mel. Lini* (**12**).

Many of the fusion cells formed undoubtedly do not function as basal cells for lack of space. Those in the upper part of the gametic tissue, immediately beneath the pseudoparenchyma, are more favor-

ably located for spore-production than those that lie more deeply in the tissue. The elongation of the fusion cell formed at the surface of the tissue is unimpeded except for the slight resistance offered by the empty, thin-walled cells of the pseudoparenchyma, while the fusion cell formed below must force its way between the closely packed gametes and fusion cells above. Sometimes they are able to do so and function as basal cells, but many were found that were apparently inhibited from so functioning by the resistance of the overlying cells.

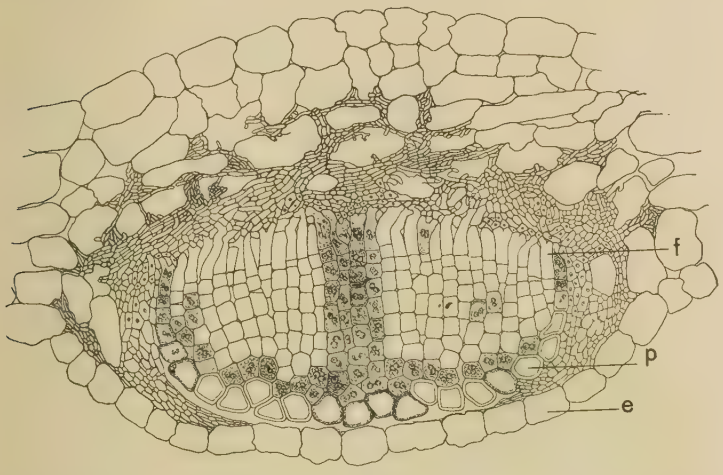

FIG. 5.—A mature aecidium of *Ur. Caladii* before the rupture of the epidermis: *e*, lower epidermis; *f*, fertile layer; *p*, peridium.

The fertile layer is deeply concave in *P. Claytoniata* and *P. Violae*, and extends about halfway up the lateral surfaces of the cup. The basal cells are more or less uneven in height, and the surface of the layer in outline has an irregular, broken appearance. The fertile layers of *Ur. Caladii* (text fig. 5) and of *P. Eatoniae* (text fig. 6) are especially broad and only slightly concave. The fertile layers of *P. Hydrocotyles* (text fig. 7) and of *P. angustata* are narrow and only slightly concave. The production of spore chains is confined to the basal surface of the cup and does not occur on the lateral surfaces. The basal cells of *P. Hydrocotyles* (text fig. 7)

form quite an even palisade and their bases converge toward a central point below. This conveys the impression that they may have arisen from a common central point or area.

Spore-production follows very rapidly after the fusions. The first spores formed are from the basal cells at the center of the fertile layer in the region where the first fusions occurred. The wave of spore-production proceeds from this center toward the borders of the fertile layer and follows closely after the fusion wave. The

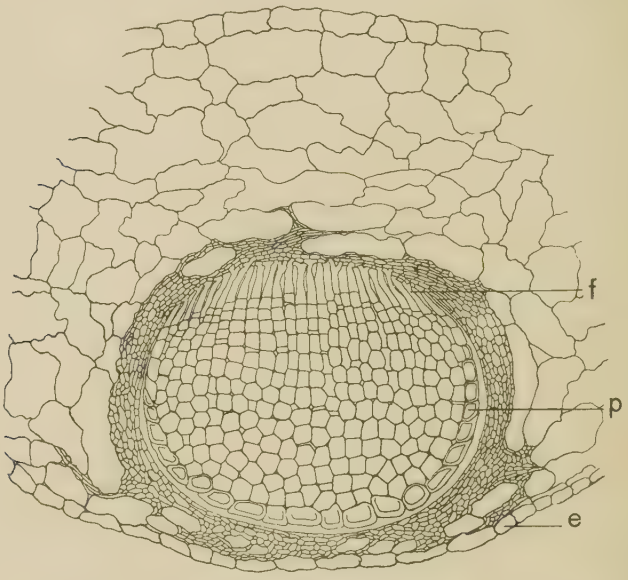

FIG. 6.—A mature aecidium of *P. Eatoniae* before the rupture of the epidermis: *e*, lower epidermis; *f*, fertile layer; *p*, peridium.

central basal cells continue to cut off spores and have usually produced a chain of 3 or 4 when the first spores are being produced from the basal cells on the extreme lateral margins of the fertile layer. The number of spores found in any one chain at this stage is dependent on the position of the basal cell with reference to the center of the fertile layer. The number decreases gradually from 3 or 4 at the center to one at the lateral margins. The surface of the spore mass is thus dome-shaped, with the highest point of the

dome at the center. It is convex in vertical section, while the surface of the fertile layer is concave. The mass of spores forms an ellipse, the long axis of which is parallel to the epidermis.

The production of spores now proceeds at a uniform rate across the entire extent of the fertile layer, and the dome-shaped roof of the spore mass is elevated or pushed toward the epidermis. The

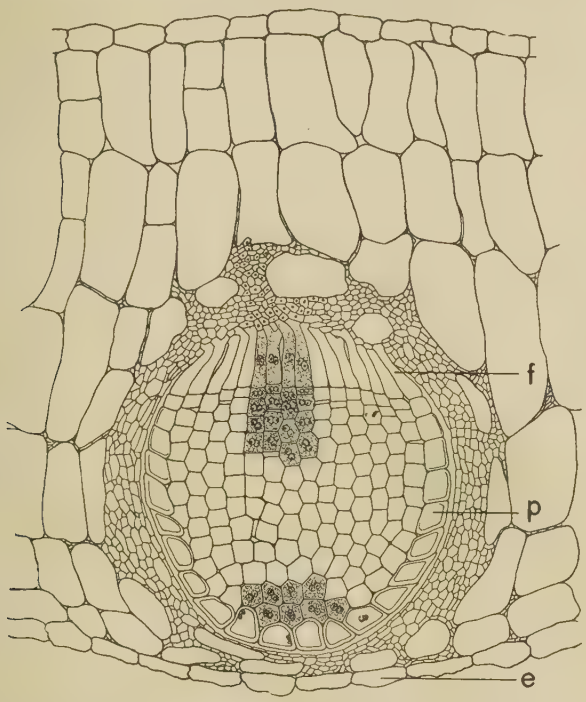

FIG. 7.—A mature aecidium of *P. Hydrocotyles* before the rupture of the epidermis: *e*, lower epidermis; *f*, fertile layer; *p*, peridium.

thin-walled, empty cells of the pseudoparenchyma apparently offer but little resistance. They are crushed and pushed upward or to one side, and either disappear entirely or remain as a thin layer of material without cellular structure. Meanwhile the leaf epidermis has been pushed up as an arch over the developing spore mass, as the result of the pressure, and when the pseudoparenchyma has

been entirely pushed away and the top of the spore mass presses directly on the epidermis, the arch is ruptured at the center.

Development of the peridium

The peridial cells make their appearance with the enlargement of the spore mass. The first peridial cells are seen at the apex of the dome-shaped spore mass, and are first distinguishable from the aecidiospores when the central spore chains are about 5 or 6 spores in length (text fig. 8). These first-formed peridial cells are produced by a metamorphosis of the apical aecidiospores of the central chains that involves an enlargement of the aecidiospore in all

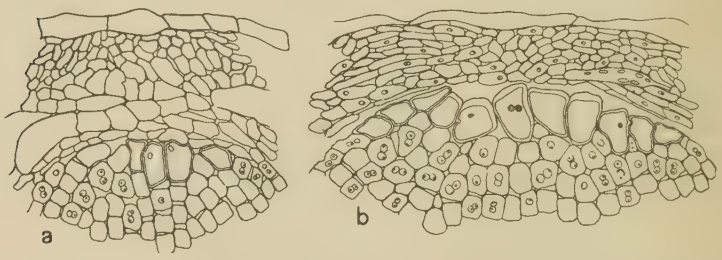

FIG. 8.—*a*, early stage in the formation of the peridium of *P. Claytoniata;* 4 peridial cells have been differentiated, all of which are apical cells of interior spore chains; *b*, a more completely formed peridium; 11 peridial cells are shown, 7 of which are from interior chains, and 4 (3 on the left and 1 on the right) are from the peripheral chains.

dimensions, a decrease in the density of its cytoplasm, and a marked thickening of its walls. The diameter of the mature peridial cell is about one and one-third times that of the mature aecidiospore, or more. The length of the cell is greater than the breadth, and its long axis is parallel to the axis of growth of the spore chain in which it is borne. This feature of the peridial cells is of some value in determining their point of origin, as will be seen later.

An early stage in the development of the peridium of *P. Claytoniata* is shown in text fig. 8, *a*. Only 4 peridial cells have been differentiated in this section. The walls of these are drawn with a double line. Three of them are apical cells of central spore chains, and the fourth is the apical cell of the fourth chain from the right-

hand border. The extreme outer border chains have as yet produced no peridial cells, and it is evident that the 4 peridial cells shown in the figure could not have been produced by them. The figure shows, further, that not all of the interior spore chains produce peridial cells, since the apices of some of these do not reach to the outer surface of the spore mass. A more complete peridium at a later stage is shown in text fig. 8, *b*. Eleven peridial cells appear in this section; 7 of these have apparently been borne on interior chains, although their connection with any one chain cannot be determined with certainty. The long axes of these cells are perpendicular to the epidermis and parallel to the axis of growth of the interior chains. The other 4 peridial cells, 3 on the left and 1 on the right, are evidently borne in the peripheral chains. It is also evident from this figure that not all of the interior chains have produced peridial cells, since there are 20 interior chains and but 7 peridial cells of interior origin.

While the peridial cell is forming, its connection with the spore chain on which it is borne is evident (text fig. 8), but with its enlargement at maturity and the changes in its position brought about by the continued elevation of the spore mass, this connection is made difficult or impossible to determine.

Not all of the peridium is formed from sterilized apical aecidiospores; its lateral walls are made up of the entire peripheral spore chains (text fig. 8, *b*). The cells of these chains are distinguishable as peridial cells when they have become the third or fourth cell from the base of the chain. The cells that make up the lateral walls of the peridium are differentiable from those that form the central arch in that they are modified aecidiospore initial cells rather than aecidiospores. They do not, except perhaps in rare cases, produce intercalary cells. Both the cytokinesis and karyokinesis of these cells are apparently inhibited by the same agent that produces the metamorphosis of the cell.

The production of peridial cells proceeds from the central point of the apical surface of the spore mass outward in all directions until a complete layer is formed over the entire apical and lateral surfaces of the spore mass. The peridium of this type of aecidium is normally but one cell in thickness. It is interesting to note,

however, that two or more layers of peridial cells are apparently formed regularly in certain species of *Peridermium* (3). When the peridium is completed over the entire outer surface of the spore mass, its subsequent enlargement is accomplished entirely by the basipetal addition of cells to its lateral walls from the peripheral basal cells.

The cytoplasm of the mature peridial cell is vacuolate and the nuclei are smaller and more compact than those of the aecidiospore. It is difficult to be sure whether they have decreased in size with the metamorphosis of the cell or whether they have not been completely reconstructed from the preceding mitosis. The latter explanation seems more probable and is supported by some observations that are not, however, conclusive. In size and general appearance they resemble the nuclei of the intercalary cells. They do not become disorganized; the chromatin masses and nucleoles stain deeply and are sharp and clean-cut. The walls stain with the orange, and a properly stained peridial cell is easily distinguished from an aecidiospore by the thickness of the wall as well as by the comparative emptiness of the cell and smallness of the nuclei. The outer side of the wall, the one next the pseudoparenchyma, is thicker than the inner or lateral sides (fig. 15). This was true to some extent of all of the forms I have studied. The nuclei are often located near the thickest part of the wall. The process of wall-thickening accompanies the decrease in the density of the cytoplasm, and the natural assumption is that the thickening is produced by the deposition of substances from the cytoplasm. No stratification appears in the walls. They may be variously sculptured, however, and the outer portion of the wall is usually marked by transverse striae.

The origin of the peridium is more easily followed in the broad cup of *Ur. Caladii* than in any of the other forms. The greater part of the peridium that is formed here before the rupture of the epidermis originates from interior spore chains. This is apparent even in mature cups, as shown in text fig. 5, which is a semi-diagrammatic representation of a stage before the rupture of the epidermis. Of the peridial cells shown here, 14 are in all probability of interior chain origin. There are about 29 interior chains.

It seems that in general the shape of the cup and the breadth of the hymenium determine the relative numbers of peridial cells of peripheral and interior origin. If the cup is shallow and the fertile layer broad, as in *Ur. Caladii*, the number of those of interior origin will be comparatively greater than when the cup is deep-seated and spherical and the fertile layer narrow, as in *P. Hydrocotyles* (text fig. 7).

So far as I am aware, no estimate of the approximate number of spores produced in an aecidium cup has been made. A comparatively accurate estimate of the number produced in the aecidium of *P. Eatoniae* was made as follows: The spore chains in median sections of 10 mature aecidia were counted. The average number found was 32. Since the cup is circular in cross-section, its radius would be 16 and the area of the cross-section 804.24. The average number of spores per chain (10), multiplied by the number of spore chains (804.24), gives a total production of 8042 spores. The estimate of 10 spores per chain is low, since the count was necessarily made before the opening of the cup, and the production of spores probably goes on for some time after this.

Wolf (**29**) has recently called attention to the production of internal aecidia of *P. angustata* on *Lycopus virginicus*. These were found principally in sections of stems and petioles. The cups are very deep-seated, and being unable to reach the epidermis discharge their spores into the parenchyma or the pith cavity. No internal aecidia appear in any of my sections of this species, as they are all of leaf tissue. I have found cases of internal spermogonia, however, in *P. Claytoniata*. These were buried at considerable depth in the parenchyma of the stem, but were normal in other respects. The spermatia had been discharged in some cases and were found in the intercellular spaces above the ostiole. Still more curious were several cases of intimate association between spermogonia and aecidia in which the spermogonium was borne in the center of an aecidium. None of the aecidia in these cases had reached the stage of sporulation, but the spermogonia had produced spermatia in abundance. The condition is evidently abnormal, but could have been construed as proof of a sexual relation between the spermogonium and aecidium by the older exponents of this view. It does

show the similar and perhaps interchangeable nature of the mycelia that produce the two fructifications.

Discussion

From the evidence gained from the study of the 6 species of aecidia treated here, the conclusion is reached that the essential processes involved in the development of the cup and caeoma types of aecidia are similar. The gametes of the two are apparently similar in origin. They are borne, in both types of sori, in perpendicular hyphae, but those of the cup are less markedly differentiated in size from the surrounding cells than those of the caeoma. Fertilization is accomplished in the cup, as in the caeoma, by complete cytoplasmic union between two morphologically equivalent gametes and produces a double, binucleated cell that later functions as the basal cell for a chain of aecidiospores. The fertile layer of both types enlarges by centrifugal growth, the first fusions occurring at the center of the gametic tissue and the last ones at the lateral margins. The breadth of the fertile layer of the cup is determinate, being limited by the breadth of the primordium and the layers of encircling hyphae on the lateral surfaces, while that of the caeoma is somewhat indeterminate, the only apparent limiting factor being the food supply.

The development of a peridium in the cup is the only sharply distinctive feature that separates the two types of aecidia. The peridium is formed as an outer layer of sterilized, differentiated cells that covers and presumably protects the spores beneath. The origin and growth of the peridium were found to conform in all essentials to RICHARDS' descriptions (**27**). It is composed of metamorphosed aecidiospores and aecidiospore initial cells that are not differentiable from the others, before their metamorphosis, except by their position on the periphery of the spore mass. The central part of the arch of the peridium is formed from the modified apical aecidiospores of the interior spore chains, while the lateral walls are formed from the undivided aecidiospore initial cells of the peripheral chains. The peridium, like the fertile layer, enlarges centrifugally. The cells at the center of the arch are first differentiated and the differentiation proceeds from this point outward in all directions

until a complete layer is formed over the entire mass. The subsequent enlargement of the peridium is accomplished by the basipetal growth of the lateral chains of peridium initial cells.

The "buffer" cells of the caeoma are homologous with the pseudoparenchyma cells of the cup, and represent a scanty production of pseudoparenchyma. The production of a peridium seems to be dependent on the presence of a considerable pseudoparenchyma, and this is in turn the result of the deep location of the primordium in the host tissue, and the deep location of the gametes in the primordium.

As I have elsewhere stated (12), in the caeoma of *Mel. Lini* the gametophoric hyphae are 3 or 4 cells long. Two sterile cells are normally produced, and though, as a rule, but one gamete is formed in each hypha, two are occasionally found. In the production of more than one sterile cell and the tendency to produce more than one gamete there is seemingly a transition toward the conditions found in the cup. The cup of *Ur. Caladii*, as found on *Arisaema*, is very much like a caeoma in appearance before the formation of the peridium. The gametophoric hyphae are 6 or 7 cells in length, and 4 or 5 of the apical cells are sterilized. If, as I have suggested, the production of a peridium is correlated with the amount of pseudoparenchyma produced, the line of separation between the aecidia without and those with a peridium should be found at some point between the caeoma of *Mel. Lini* with 2 sterile cells, and the cup of *Ur. Caladii* with 4 or 5. A careful study of the more deeply seated caeomas may perhaps reveal a more or less marked differentiation of the outermost spore layer. Those species of cupulate aecidia with evanescent peridia are perhaps most nearly like the deep caeomas.

The study of the origin of the peridium that is found in the uredosorus of certain genera should prove of considerable interest, but apparently no such study has ever been made and no data are available. According to ARTHUR'S treatment of the Uredinales in the *North American flora* (2), 6 genera of the family Uredinaceae have uredosori with peridia: *Pucciniastrum*, *Melampsoridium*, *Hyalopsora*, *Uredinopsis*, *Melampsoropsis*, and *Cronartium*. *Melampsoropsis* has catenulate uredospores, while

those of the remaining 5 genera are reported as pedicellate. A peridium of the type found in the aecidium cup is to be expected in a deep-seated uredosorus of catenulate spores, but is scarcely to be looked for in a sorus of pedicellate spores, where the supposed protective function is normally performed by paraphyses.

No central organs ("fertile hyphae") or multinucleated cells were found in any of the species of aecidia studied, and it is therefore concluded that these do not necessarily occur in aecidia of the cup type. While this by no means precludes the production of multinucleated cells in other species, it is evidence that these are not necessary for the development of the centralized structure of the cup. It is perhaps possible that the multinucleated cells found by OLIVE in certain cupulate aecidia are the result of multiple cell fusions. The presence of tricellular fusions and trinucleated and quadrinucleated aecidiospores in several of the species I have studied shows that pluricellular fusions are rather common in the cup as well as in the caeoma.

The aecidium cup, from the evidence presented here, is not to be regarded as an organ with a centralized development like that of the ascocarp, as DEBARY and others have considered it, but is seen to be merely a remarkably unified colony of individual gametophores. The presence of a central organ is no more necessary to the development of the cup than the caeoma. The existence of uredosori with catenulate spores, that arise from sporophytic mycelium and hence cannot be considered the product of central organs, is further evidence that a seemingly centralized fructification may be developed from a colony.

The presence of trinucleated spores in various rust sori has been noted by a number of investigators, and there seems to be no question that such spores, when found in the aecidium or the teleutosorus of the micro-forms, are the products of tricellular fusions. The germination of a trinucleated spore would be highly interesting to observe. HOFFMAN believes that one of the nuclei in trinucleated spores of *Endophyllum Sempervivi* degenerates, but has no convincing proof of this.

As previously noted, branching basal cells have been found in the aecidium of *P. Falcariae* by DITTSCHLAG and in the sorus of

Endo. Sempervivi by HOFFMAN. Although I have found no such cells in any of the aecidia included in this study, I have found a single case of a basal cell that seems to have given rise to a branch in the aecidium of *Aecidium Dicentrae* Trel. Only a few sections of this form have been examined, and hence no estimate can be made as to the prevalence of the condition. In the single case seen, the second basal cell seems to have arisen as a lateral branch of the primary basal cell, and the opening between the two has not been closed by wall-formation. Both basal cells have produced a chain of spores.

HOFFMAN compares these branching basal cells to the basal cell of the primary uredosorus as described by CHRISTMAN. The two cases, however, are not at all comparable. The basal cell of the primary uredosorus gives rise to successive stalked spores that originate as buds on the upper surface of the cell. The comparison is made, therefore, between a bud that produces a stalked spore in one case and a basal cell in the other. As CHRISTMAN has pointed out, the homology lies between the primary uredospore and stalk cell and the aecidiospore and intercalary cell. The homology is also extended to include the basal cells of the two sori.

According to CHRISTMAN, the basal cell is the true morphological unit, and the basal cells of the different sori are to be considered as homologous. This, however, involves the difficulty, which CHRISTMAN recognized, that the basal cells of the uredosorus and teleutosorus are not the outgrowth of fusion cells, as are those of the primary uredo and aecidium, but are borne on a mycelium of binucleated cells. The basal cell of the micro-forms is also without doubt to be homologized with that of the aecidium, since OLIVE's work on *P. transformans* and that of WERTH and LUDWIGS on *P. Malvacearum* indicate that the basal cell of these forms is the outgrowth of a fusion cell.

The evident homology between the primary uredospore and the aecidiospore is used by CHRISTMAN as a strong argument that the eu-type of rust with catenulate aecidiospores cannot be considered as a primitive type, but is rather a highly specialized and derived type. If the homology is to be accepted, and it seems to have been very generally, the catenulate method of aecidiospore-production

must have been derived from the pedicellate, and the primary uredo is therefore more primitive than the aecidium. For, as he argues, the intercalary cell is thus seen to be in reality a stalk cell that has been necessarily shortened by the catenulate method of spore-production and persists as a vestigial cell. If the aecidium, however, is considered the primitive sorus and the uredo the derived form, the intercalary cell must be considered a functionless structure that persists and is later modified into a structure of value to the organism in the stalk of the uredospore.

In view of this convincing explanation of the nature of the intercalary cell, one can scarcely agree with GROVE (13) that *Endo. Sempervivi* is to be considered as a representative of the primitive type of rusts. The fact that the spores of this species are borne in a complex aecidium-like fructification, surrounded by a peridium and accompanied by intercalary cells, makes its acceptance as a primitive type extremely difficult. To all morphological appearances the spores are aecidiospores and the sorus an aecidium. The spores, however, function as teleutospores, as HOFFMAN has conclusively shown. GROVE'S acceptance of *Endophyllum* as a primitive type and his relegation of the micro-forms to the position of reduced types seem inconsistent when the relative complexity of the two types of sori are considered. It certainly seems more logical to consider *Endophyllum* a reduced form, as the gametophytic generation of a former eu-heteroecious or autoecious species that has dropped the uredo and teleuto stages, with the assumption of a teleutosporic method of germination by the aecidiospore, and to regard the micro-forms as the more primitive and ancestral. The assumption of the teleutosporic method of germination by an aecidiospore is not a difficult conception, since nothing more than the fusion of nuclei in the spore should be necessary for its accomplishment.

It is interesting to note that KUNKEL (17) has recently discovered a companion form to *Endo. Sempervivi* in the common orange rust of the blackberry, *Caeoma nitens*. The life history of the two species is practically identical, seemingly, but the sorus of the latter is a caeoma, while that of the former is an aecidium cup. *Caeoma nitens*, therefore, should offer a much better ancestral type for

GROVE's argument than *Endo. Sempervivi* because of the relative simplicity of the sorus, but the presence of intercalary cells, on the other hand, is a strong argument against its acceptance as such.

GROVE advances the perennial nature of the mycelium of *Endo. Sempervivi* as further proof of the primitive nature of the fungus. The perennial nature of the mycelium seems to me, however, a highly specialized condition. A more desirable relation from the standpoint of the continued welfare of the fungus is difficult to conceive. The fungus thus becomes independent of the chance association of hosts for its propagation. A decrease in the number of spore forms is perhaps to be expected with the increased efficiency of the perennial mode of life. The necessity for the production of resting spores is done away with, as well as the production of repeating spores for extensive propagation. A perennial mycelium is also found in certain of the short-cycled micro-forms, for example *P. fusca* and *P. Adoxae*, and it seems, therefore, that this condition cannot be advanced as proof of either a primitive or a derived condition. The efficiency of the perennial gametophytic mycelium may be one reason why the gametophyte has maintained the supremacy and these micro-forms have persisted as such.

The remarkable cases of correlation between the teleutospores of certain micro-forms and those of certain eu-heteroecious forms that have been cited by FISHER (11) and others are certainly strong evidence of a phylogenetic relation between the two groups. Further study of this phase of rust morphology will doubtless bring to light many groups of correlated species with life cycles of various lengths, and will in all probability settle many of the problems as to the origin and development of such types. The most reasonable view of the phylogeny of the rusts seems to me that of DIETEL as amplified by CHRISTMAN and OLIVE. This view regards the micro-forms as the most primitive forms, from which the brachy, opsis, and eu-forms have been derived by the lengthening of the sporophyte generation and the intercalation of aecidiospores and uredospores.

This work was conducted under the direction of Professor R. A. HARPER, to whom I am indebted for many helpful suggestions and criticisms.

Summary

1. The essential features in the development of the cup are similar to those found in the development of the caeoma. The initial hyphal mass, or primordium, is formed by hyphae growing radially toward the center of the future cup.

2. The cup is more deeply seated and produces a greater number of sterile cells and gametes to each gametophoric hypha. The gametes form a fertile layer two or more cells in thickness. The sterile cells that form the pseudoparenchyma of the cup are homologous with the "buffer" cells of the caeoma.

3. Sexual cell fusions, by the breaking down of the cell walls between two equal gametes, were found in 6 additional species of cupulate aecidia, namely *Ur. Caladii*, *P. Claytoniata*, *P. Violae*, *P. Hydrocotyles*, *P. Eatoniae*, and *P. angustata*. Although the actual fusion stages were not seen in the last named species, the presence of two-legged basal cells is evidence that the fusions are of the same type as those found in the other species. No central organs ("fertile hyphae") or multinucleated cells were found. The organization of the cup, therefore, is merely that of a remarkably unified colony of gametophores.

4. Triple cell fusions were observed in *P. Claytoniata* and *P. Violae*, and trinucleated aecidiospores were frequently found in both of these species and in *Ur. Caladii*. Several quadrinucleated aecidiospores and a chain of quadrinucleated cells were found in *P. Claytoniata*.

5. The first fusion cells are formed at the center of the gametic tissue, and the subsequent ones are formed on all sides of this center in centrifugal order, until the lateral borders of the aecidium are reached.

6. The fusing cells may have their long axes in general in the long axis of the cup, for example *P. Claytoniata*, *P. Violae*, *P. Hydrocotyles*, and *Ur. Caladii*, or tangential to its curved basal surface, for example *P. Eatoniae*.

7. The presence or absence of a peridium is a natural but not very fundamental distinction between the aecidium cup and the caeoma. The production of a peridium is correlated with the deep location of the cup and the extensive formation of sterile cells.

8. As has long been known, the peridial cells are metamorphosed aecidiospores and aecidiospore initial cells. The central arch of the peridium is formed from the apical aecidiospores of the interior spore chains and the lateral walls from entire peripheral spore chains. The first peridial cells are produced at the center of the arch and the peridium enlarges from this point centrifugally until the bases of the lateral walls are reached. Its subsequent enlargement is by the basipetal growth and sterilization of the peripheral spore chains.

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EXPLANATION OF PLATES I AND II

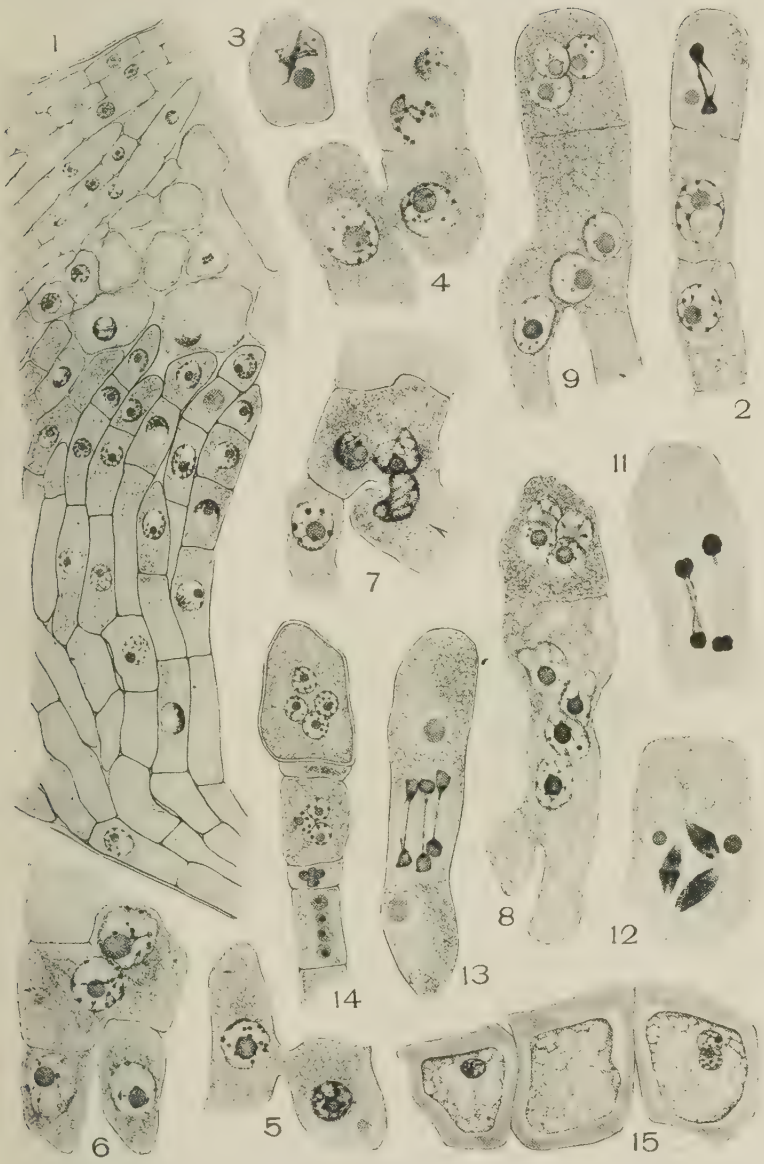
All of the figures were drawn with the aid of the camera lucida, and, except where otherwise mentioned, at a magnification of 1140 diameters. They are all placed in their natural position in the sorus, with the top of the page representing the direction of the leaf epidermis.

Puccinia Claytoniata

FIG. 1.—A section through the lateral border of the cup before the cell fusions; 6 layers of encircling hyphae are seen at the top, below these are the sterile cells of the pseudoparenchyma and below these the perpendicular gametophoric hyphae; all of the cells are uninucleated; ×570.

FIG. 2.—A more highly magnified view of the tip of a gametophoric hypha in a young aecidium; the nucleus of the apical cell is shown in the anaphase of mitosis.

FIG. 3.—An earlier stage of mitosis in a uninucleated cell.



FROMME on AECIDIUM CUP

FROMME on AECIDIUM CUP

FIGS. 4 and 5.—Two early stages of cell fusions.

FIG. 6.—A completed cell fusion.

FIG. 7.—A tricellular fusion.

FIG. 8.—A two-legged basal cell and above it an aecidiospore initial cell; 4 nuclei are found in both cells as the result of the preceding conjugate divisions; ×900.

FIG. 9.—A trinucleated basal cell and aecidiospore initial cell.

FIG. 10.—Early stage of conjugate mitosis in an aecidiospore initial cell.

FIG. 11.—Anaphase of conjugate mitosis in a fusion cell.

FIG. 12.—Simultaneous division of three nuclei in a fusion cell.

FIG. 13.—A later stage than the preceding; the elongated spindles lie parallel with each other and the long axis of the fusion cell.

FIG. 14.—A chain of quadrinucleated aecidiospores and intercalary cells; ×570.

FIG. 15.—Three peridial cells from the central region of the peridium; ×570.

Uromyces Caladii

FIGS. 16 and 17.—Two cases of completed cell fusions.

FIG. 18.—A tricellular fusion.

FIG. 19.—A two-legged basal cell.

Puccinia Violae

FIG. 20.—A very early stage of cell fusion in which the fusion pore is still very small.

FIG. 21.—A completed cell fusion.

FIG. 22.—A two-legged fusion cell.

Puccinia Hydrocotyles

FIG. 23.—Three cases of cell fusion within a small area; the borders of two cells of the pseudoparenchyma are shown at the top of the figure.

Puccinia Eatoniae

FIG. 24.—Fusion between two cells in horizontal lying hyphae near the base of the sorus; the base of a two-legged fusion cell lies just above the fusing gametes.

FIG. 25.—A two-legged fusion cell that has bent up at a sharp angle and elongated in the direction of the epidermis.

Puccinia angustata

FIGS. 26 and 27.—Two-legged basal cells.